INVERTASE ACTIVITY IN ADULT *AEDES AEGYPTI* MOSQUITOES

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In connection with studies of proteolytic digestion in the *Aedes aegypti* msoquito (Fisk, 1950; Fisk and Shambaugh, 1952; Shambaugh, 1954) a technique was devised for measuring the activity of invertases and amylases in adult mosquitoes. The results of this study are reported here.

As is well known, most adult female mosquitoes feed primarily on blood, which is taken directly into the midgut, and secondarily on nectar, exposed fruit juices or other sweetened liquids, which pass first to the diverticula for temporary storage and then, gradually, back through the midgut. The small paired dorsal diverticula and the larger ventral diverticulum or crop are all blind tubes consisting of thin transparent sacs covered with a syncitium of muscles sufficient to provide a high degree of elasticity and some mobility. No secretory epithelium has been found, so that any digestive enzymes noted in the diverticula are presumed to have been secreted elsewhere.

METHODS

The methods of securing, feeding, incubating, dissecting, and preparing homogenates from adult female *Aedes aegypti* mosquitoes have already been adequately described (Fisk and Shambaugh, 1952). The methods of enzyme analysis for invertases or amylases were identical except for substrate and employed a modification of the 3,5-dinitrosalicylic acid technique of Sumner (1925). This is a colorimetric test for reducing sugars which has been shown by Walker and Reisinger (1933) to detect as low as one microgram of reducing sugars in a 0.2 μl. sample of glomerular urine. This corresponds to a 0.5 percent concentration of reducing substances. With larger samples, more dilute solutions can be determined.

The dinitrosalicylic acid reagent, which remained useful for a month or more in a refrigerated, glass-stoppered bottle, was prepared as follows: Ten grams crystallized phenol was added to 22 ml. 10 percent NaOH and diluted with water to 100 ml. Sixty-nine milliliters of this alkaline phenol solution was then added to 6.9 g. $NaHSO_3$, 300 ml. 4.5 percent NaOH, 255 g. Rochelle salts, and 880 ml. 1 percent 3,5-dinitrosalicylic acid.

Invertase determinations were made in the following manner: The dissected viscera were removed from the deep-freezer, homogenized in Levy's saline, and diluted with saline to 1.6 ml. Three 0.5 ml. aliquots were taken from the homogenate, one being placed in a boiling water bath, and the other two in the deep-freezer. After 5 min. the aliquot was removed from the water bath and placed in the deep-freezer with the others for 5 min. Then the substrate, 0.5 ml. 2.5 percent sucrose in a phosphate buffer of pH 6.5, was added and stirred. The brei was incubated an hour at 35° C. Incubation was stopped by emptying the brei into a tube containing 2 ml. of dinitrosalicylic reagent and placing it in a boiling water bath for 5 min. At the same time a reagent blank prepared from 1 ml. water and 2 ml. reagent was given the same treatment. All the tubes were then cooled 3 min. in running water. Aliquots of 0.5 ml. were diluted to 10 ml. and read in a Klett-Summerson photo-electric colorimeter using the green (540 mμ) filter.

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Amylase determinations were made in the same manner, but with a change of substrate to 0.5 ml. portions of 1.5 percent soluble starch plus 0.5 ml. phosphate buffer, pH 6.5.

RESULTS AND DISCUSSION

The three possible sources of digestive enzymes considered were the salivary glands, the diverticula (including the crop), and the midgut. Metcalf (1945) found amylase, protease, and lipase to be absent from the salivary glands of *Anopheles quadrimaculatus*. Our preliminary tests showed invertase to be absent from the salivary glands, but present in both midgut and crop homogenates. Separate invertase determinations were then made for midgut and diverticula tissues from blood-fed and from sugar-fed mosquitoes. The results of these four series of tests are shown in table 1.

TABLE 1

Comparative invertase activities, expressed in colorimeter units, of diverticula and midgut homogenates respectively from blood-fed and from 5% sucrose-fed female **Aedes aegypti.**

Interval of Time After Feeding	Blood-fed Mosquitoes†		5% Sucrose-fed Mosquitoes	
	Diverticula	Midguts	Diverticula	Midguts
Unfed	70 (3)	108 (2)	70 (3)	108 (2)
½ hr.	75 (2)	224 (2)	20 (2)	85 (2)
1 hr.	64 (3)	155 (3)	33 (2)	108 (4)
2 hr.	24 (1)	335 (1)	34 (2)	106 (2)
4 hr.	(0)	99 (1)	36 (3)	92 (2)
8 hr.	35 (1)	131 (1)	14 (2)	102 (2)
12 hr.	58 (1)	116 (1)	18 (2)	93 (2)
24 hr.	40 (2)	125 (2)	27 (1)	84 (1)

†The values given are averages of the number of tests indicated in parenthesis, each test consisting of duplicate runs and a boiled blank on material from 20 mosquitoes.

Tests for amylase run on single homogenates of salivary glands, diverticula, and midguts of unfed mosquitoes were all negative. Although further tests were not run, it is possible that significant amylase activity could be demonstrated in crops or midguts of blood-fed mosquitoes.

The data in table 1 indicate appreciable amounts of invertase in both midguts and diverticula of unfed or starved mosquitoes. This residual activity is lower for the diverticula and remains so following feeding, in fact it decreases within half an hour following a syrup meal and within two hours after a blood meal. The comparatively rapid decrease after sugar feeding is probably due to a depletion of the enzyme by its substrate, as described by Day and Powning (1949) for the roach, but the same explanation cannot apply to the apparent decrease after a blood meal since the blood is not found in the diverticula nor does it contain a substrate for invertase.

Invertase activity in the midguts shows a definite increase following a blood meal. This stimulation, the greatest shown in any of the tests reported in this paper, lasts somewhere between two and four hours, a short time compared to the protease stimulation after a blood meal which is at its peak at about 18 hr. after feeding (Fisk and Shambaugh, 1952). After a meal of 5 percent sucrose, invertase activity in the midguts shows little change. If the small differences shown for this series in table 1 can be interpreted as indicative of actual trends, they would show an initial depletion (similar to that noted in the diverticula under the same circum-

stances), followed by a slight increased activity past the two hour interval then a return to the residual value.

The stimulation of invertase activity in *Aedes aegypti* stands in contrast to the stimulation of protease in the same species. Invertase activity is stimulated very little by its proper substrate, sucrose, but very definitely by that primary foodstuff of the female mosquito, blood. Protease activity was found to be stimulated profoundly by its proper substrate, blood, but only briefly and in small amount by sugar syrup. It is apparent that secretions of both invertase and protease are stimulated by factors, possibly identical factors, in the blood meal.

SUMMARY

A quantitative colorimetric method is described whereby the invertase or amylase activities of insect tissues homogenates can be determined. The technique measures microgram quantities of reducing sugars, using a 3,5-dinitrosalicylic acid reagent.

Tests with unfed female *Aedes aegypti* mosquitoes gave negative results for amylase in the midguts, and diverticula and salivary glands and for invertase in the salivary glands. Significant invertase activity was found in midgut and diverticula tissues of unfed or starved individuals.

Determinations were run comparing the invertase activities of midguts and diverticula at various intervals after feeding on blood or on 5 percent sucrose.

At all intervals tested, invertase activity was highest in the midgut tissues, agreeing with morphological evidence that the enzyme is secreted in the midgut, not the diverticula.

Definite increases in invertase activity of less than four hours duration occurred in the midgut following blood-feeding, while the changes following sucrose feeding were relatively slight.

The diverticula, on the other hand, showed decreases in activity following the feeding of either food.

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